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THE SCENT-PRODUCING ORGAN OF THE HONEY BEE.

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INTRODUCTION.

Bee keepers well know that bees have an odor, but they do not know how the odor is produced, nor do they know the rôle played by the various odors of the honey bee. It is reported that Nasonoff first described the morphology of the scent-producing organ of the honey bee. His original work in Russian cannot be had here, but, according to Zoubareff (1883), Nasonoff did not describe the structure of this organ as seen by the writer, and he suggested that the gland cells of the organ produce perspiration. Sladen (1902) called this organ a "scent-producing organ," but did nothing more than to describe the articular membrane between the fifth and sixth abdominal terga of worker bees.

This paper deals entirely with the morphology of the scent-producing organ. The work dealing with the odors produced by this organ and the significance of these odors will be reported separately.

Fresh material was stained slightly with a weak solution of methylin green, and the cells were studied while still alive. Material was also fixed in Carnoy's fluid (equal parts of absolute alcohol, chloroform, and glacial acetic acid, with corrosive sublimate to excess). The double method of embedding in paraffin and celloidin was employed. Sections were cut 10 micra thick and they were stained with Ehrlich's hämatoxylin and eosin, and with safranin and gentian violet.

1. STRUCTURE.

Sometimes when a worker honey bee, that is fanning, is carefully observed, a transverse white stripe near the end of the abdomen may be seen. This white stripe (fig. 1, *ArtM*) is the articular membrane between the fifth and sixth abdominal terga (propodeum not counted). It is visible only when the last abdominal segment is bent downward. The anterior half of this membrane is folded under the posterior edge of the fifth abdominal tergum, making a pouch or canal (fig. 1, *Can*). The canal encircles about one half of the abdomen and terminates on either side of the abdomen just above the articulation of the

tergum and sternum (fig. 1, *ECan*). The diameter of the canal is greatest at the median line of the abdomen and gradually diminishes to zero at each end.

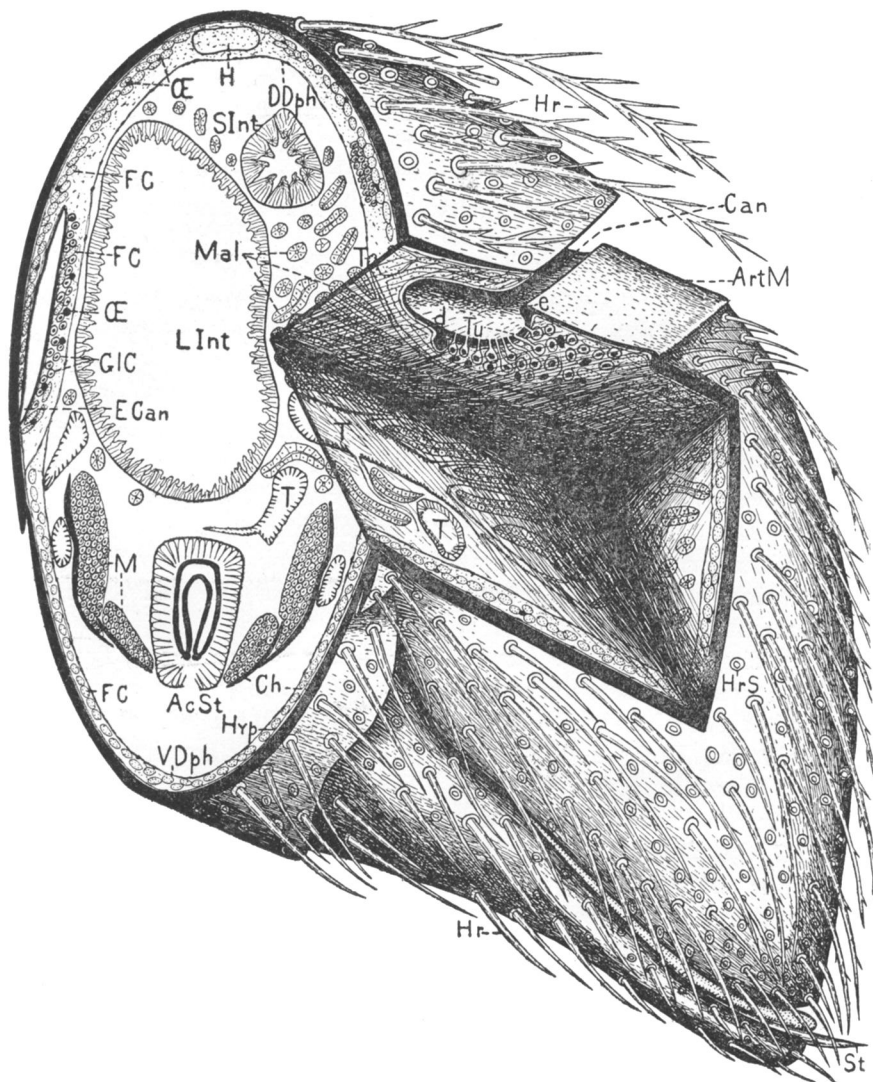


Fig. 1.—Diagram of a transverse-longitudinal view of end of abdomen of a worker honey bee, showing the internal anatomy of the fifth and sixth segments, and also the scent-producing organ composed of the articular membrane (*ArtM*), the canal (*Can*), chitinous tubes (*Tu*) and gland cells (*GIC*). The last segment is bent downward more than ever seen in the living bee. That is, in the living bee only the part marked *ArtM* is seen externally and the canal (*Can*) is never seen.

Fig. 2 is a diagram of the articular membrane, removed from the abdomen and spread out flat under a low-power lens. This membrane in the living bee is shiny and appears to be covered with a transparent liquid. The anterior margin of the membrane is bordered by small barbed hairs (fig. 2, *a*) on the fifth tergum, and the posterior margin is bordered by smaller spinelike hairs (fig. 2, *b*) on the sixth tergum. The chitin of the posterior portion (fig. 2, *PostP*) of this membrane is thinner than is the chitin of the tergum, but it is strengthened near its centre by a narrow and heavy vein (figs. 1 and 2, *e*), and at its anterior margin there is a heavier and much wider vein (figs. 1 and 2, *d*).

The chitin of the anterior portion (fig. 2, *AntP*) of the membrane is much thinner than is that of the posterior portion. It is quite flexible and for this reason may be easily folded to form the canal. Instead of it being perfectly smooth, as is the posterior portion of the membrane, its surface is covered with innumerable minute, narrow, groove-like indentations. These may be comparatively long or short, bent, tortuous, or straight and seemingly extend half way through the chitin. The small lines in fig. 2, *c*, represent their arrangement and fig. 3 represents a few of them seen under a high magnification. Of course, they are not slits passing entirely through the chitin, but they are grooves and pass about one half through the membrane.

Looking through the chitin of the posterior portion (fig. 2, *PostP*) of the articular membrane, at a deeper focus, may be seen many round cells, each of which has a tube that runs to the surface of the membrane. In fig. 2, 115 of these tubes with cells are shown, but in all there are from 500 to 600. The majority of the tubes have exits in the chitin between the two heavy veins (figs. 1, Plate XIX, and 2, *d* and *e*), but none of them has an exit in the chitin of the anterior portion (*AntP*) of the membrane. The place where these tubes empty is best seen in fig. 1, *Tu*. It is thus seen that the tubes unite with the posterior wall of the canal which is formed by the heavy chitin between *d* and *e* in figs. 1 and 2. The bottom and anterior wall of the canal are formed by the anterior portion of the articular membrane.

Fig. 4 represents four of the cells and several of the tubes seen under a high-power lens. *a* represents comparatively thin and almost transparent chitin; *b* is a narrow, thick, and yellow band of chitin; *c* is a thick, semitransparent band of chitin; *d* is a wide, thick, and opaque band of chitin; *e* is thick, semitransparent chitin. It is

thus seen that the cells lie beneath the thinnest portion of the chitin belonging to the posterior part of the articular membrane, and that their exits lie in the thickest portion of this chitin. A transparent area (Plate XIX, fig. 4, *Amp*) was seen in many of the cells, and a tube (fig. 4, *Tu*) runs into each of these areas.

In order to study these cells in a living state more carefully, the articular membranes including the tissues adhering to them were removed from worker bees. This material was placed on a slide in a weak solution of methylin green. The cells adhering to the chitin were teased apart and a few of them with their tubes were separated from the mass of cells and chitin. Such a treatment, however, almost always pulls the internal ends of the tubes out of the cells, whereupon the transparent areas disappear immediately. The tubes are then attached only at their peripheral ends.

The cells vary considerably in size. They are either spherical or ovoid in shape. Fig. 5 represents one of the largest ovoid cells. It is typical and was drawn with the aid of a camera lucida while still alive, being stained very slightly with methylin green. The large nucleus has a heavy wall, and it stands out conspicuously. The nucleoli with heavy walls stain green. The cytoplasm in the centre of the nucleus has a faint green color, while that near the periphery of the nucleus is semitransparent. The wall of the cell is thin. The cytoplasm of the cell is more or less transparent. It is granular and appears to have innumerable minute clear spots (*CLS*). In the broader end of the cell lies the ovoid, transparent area, which may be called the ampulla (*Amp*). The tube (*Tu*) terminates at the centre of the ampulla. The ampulla seems to have many lines or streaks which radiate from the periphery toward the centre, and these radial streaks (*RadStr*) stop short of the centre and leave a perfectly transparent, ovoid area (*TrA*) at the centre of the ampulla.

Judging from the structure of these cells, we must call them gland cells, but when observed hurriedly they may be mistaken for œnocytes. As a rule, the œnocytes are smaller than the gland cells, but nevertheless many of them are as large as many of the gland cells. Only a few œnocytes may be found among the mass of gland cells, but they are quite abundant on all sides of the gland cells. Fig. 6 represents a typical large œnocyte, still alive and stained slightly with a weak solution of methylin green. The following may be used to distinguish a gland cell from an œnocyte. An œnocyte is never connected with a tube. It never has an ampulla.

Its cytoplasm is less granular. It is always partially, and sometimes almost totally, filled with globules (*Glo*).

Chiefly on account of its size, a fat cell should never be mistaken for a gland cell. Fat cells are always larger, and sometimes several times larger, than these gland cells. They are found on all sides of the mass of gland cells, but seldom among them. Their structure is similar to that of oenocytes, but the globules are much larger, more conspicuous and are so abundant that the nucleus is scarcely visible. Fig. 7 represents a small fat cell, still alive and stained slightly with a weak solution of methylin green.

To ascertain if the tubes connecting the gland cells with the chitin are composed of chitin, articular membranes removed from the abdomens of workers were placed a few hours into a saturated solution of caustic potash. When all the adhering tissues had disintegrated, the membranes were cleaned with water and a pencil brush. In all cases the tubes were left attached to the membranes. This proves that they are chitinous. To determine how they terminate in the articular membrane, one of the membranes treated with caustic potash was sectioned. The sections show that the canal of the tube opens freely to the exterior (fig. 8, *CanTu*).

Judged by the morphology, we may reasonably conclude that the gland cells secrete a volatile substance throughout their cytoplasm. This substance collects in the ampulla which serves as a reservoir, and from the ampulla it passes through the chitinous tube to the exterior where it runs into the canal. The groove-like indentations in the chitin forming the canal may serve two purposes—(1) to give more flexibility to the chitin, and (2) to retain the volatile secretion and help prevent a too rapid evaporation of it. As long as the abdomen is straight, the canal is well protected and the liquid cannot evaporate rapidly, but when the abdomen is considerably bent, the entire canal is more or less exposed to the outside air.

2. ORIGIN OF GLAND CELLS.

The scent-producing organs of several 15-day-old worker pupæ (counting from the time the eggs were laid) were sectioned. At this stage the chitin (fig. 9, *Ch*) is just beginning to be formed, and the hypodermis (fig. 9, *Hyp*) is very thick. The fat cells (fig. 9, *FC*) are also not yet completely differentiated. The hypodermal cells (fig. 9, *HypC*) are long and slender. Most of them near the place where the wide and heavy vein (figs. 2, *d*, and 9, *v*) is later formed, break loose from the hypodermal layer and migrate backward

till the majority of them lie posterior to the heavy vein. In fig. 9, *a*, a row of them has broken loose from the hypodermal layer and they are assuming the ovoid shape. At fig. 9, *b*, they are a little farther advanced. In the 16-day-old stage the gland cells (fig. 10, *GIC*) are much larger and lie just back of the heavy vein (fig. 10, *v*). Now the chitinous tubes (fig. 10, *Tu*) are formed and they are connected with the gland cells.

3. ORIGIN OF CHITINOUS TUBES.

In the 15-day-old stage may occasionally be seen hypodermal cells having processes. Such cells lie at the place where the chitinous tubes later appear. One of these cells (fig. 11*a*) has a large and conspicuous nucleus. The growing point of the process appears to have no cell wall. Twelve hours later the hypodermal cells (fig. 11*b*) forming the tubes have become much smaller, no doubt because of the formation of long processes. It seems that the more the processes grow in length, the more the cells diminish in size. Each hypodermal cell, therefore, must serve as a storehouse for building a tube. When the process is far advanced, its cytoplasm probably begins secreting a substance which in a short time is transformed into the chitinous tube. In fig. 11*b* the tube is developed and it is connected with the exterior, but the cytoplasm surrounding the tube has not yet disappeared. In a little later stage (fig. 11*c*); the cytoplasm surrounding the tube has all disappeared except a small process of the cell. The tube is now connected with a gland cell.

4. DEVELOPMENT OF GLAND CELLS.

As already stated, the gland cells were originally hypodermal cells (fig. 9, *a* and *b*) which migrated from the hypodermal layer. This migration occurs in worker pupæ 15 days old. In 16-day-old worker pupæ these cells (fig. 10, *GIC*) are three or four times as large as they are in the 15-day-old stage and they begin to resemble true gland cells. In the 17-day-old stage they are still larger (fig. 12, *GIC*). Their nuclei are extremely large and stain less densely than does the cytoplasm with Ehrlich's hämatoxylin and eosin. By the nineteenth day the gland cells (fig. 13, *GIC*) have enlarged but little since the 17-day-old stage. In 21-day-old worker pupæ (age at which they emerge from their cells) the gland cells (fig. 14, *GIC*) seem to be perfectly developed in all respects, except they are only about two-thirds the size of the gland cells (fig. 15, *GIC*) in old worker bees.

It is quite possible that the gland cells never function until the bee has emerged. It seems reasonable, therefore, to regard the rapid growth which takes place in these cells after the bees have emerged to the fact that the gland cells suddenly begin to function.

5. SCENT-PRODUCING ORGAN OF QUEEN.

The articular membrane between the fifth and sixth abdominal terga of a queen honey bee is never visible externally, except at the instant when she bends her abdomen to sting an object beneath her. Several of these articular membranes of queens were excised and were examined in the same manner as already related for those of workers. Gland cells and chitinous tubes are present in the same position and arrangement as they are in workers.

All the other articular membranes between the abdominal terga in queens and workers were examined, but no chitinous tubes nor gland cells were found.

The gland cells (fig. 16a) in adult queens are at least one-third larger than are those in adult workers (fig. 15, *GLC*) and in fixed and stained sections they have the same structure. The gland cells (fig. 16b) in pupæ of queens also have the same structure as those in pupæ of workers.

6. DOES A DRONE HAVE A SCENT-PRODUCING ORGAN?

All the articular membranes between the abdominal terga of several drones were excised and carefully examined. At no time did the writer ever find chitinous tubes attached to any one of these membranes and he never saw any cells adhering to the membranes which resemble the gland cells already described. This does not mean that drones do not have any scent-producing organs, because other parts of the body and all the appendages were not examined for glandular structures. Scent-producing organs in males of several other insects have been described, so that such an organ may still be found in drones.

Sometimes when the abdomens of young drones are slightly squeezed, a very thin and whitish liquid may be seen on the abdominal articular membranes. At other times a clear liquid may be observed on the articular membranes, particularly on those between the fourth and fifth, and fifth and sixth abdominal terga. This clear liquid has a saline taste, and in this respect resembles the blood of drones.

7. DISCUSSION.

A discussion of all the literature available pertaining to the scent-producing organs of insects has been prepared, but since such a long discussion cannot be presented here, only a brief outline will be given.

A review of the literature shows that the substance produced by any scent-producing organ is secreted by unicellular glands which as far as known are modified hypodermal cells. For description, scent-producing organs may be divided into five types based on their devices for disseminating the odor and for storing the secretion as follows: (1) No special device for disseminating the odor or storing the secretion; (2) gland cells associated with hairs and scales as a means of scattering the odor more effectively; (3) "evaginable" sacs lined with hairs connected with gland cells as a device for storing and distributing the odor; (4) articular membranes serving as pouches for storing and preventing a too rapid evaporation of the secretion; (5) specialized tubes and sacs acting as reservoirs for storing and discharging the secretion.

The first type is the simplest of all five types. It is best represented as unicellular glands uniformly distributed over the entire body surface as found in some beetles (Tower, 1903). In the beetles *Dytiscus* and *Acilius* unicellular glands lie just beneath the hypodermis between the head and tergite of the prothorax (Plateau, 1876). In the blister beetle, *Meloe*, are found unicellular glands beneath the hypodermis on both sides of the femoro-tibial articulations (Berlese, 1909). These gland cells are similar in structure to those of the honey bee. Beneath the femoro-tibial articulation in *Camponotus* and the tibio-tarsal articulation in *Formica*, Schön (1911) found unicellular glands. Beneath the hypodermis of the caruncles of the Indian roach, *Corydia*, lie unicellular glands, also similar to those of the bee (Klemensiewicz, 1882). In this type of scent-producing organ the secretion passes through the chitinous tubes to the exterior where it spreads over the surface of the chitin surrounding the exits of the tubes.

In regard to spreading the secretion over a wider area, the second type is much more highly developed than is the first type. This is accomplished in most cases by the secretion spreading over the surfaces of many large hairs arranged in tufts which may be expanded into a fan-shaped figure. The hind tibiae of the male moth *Hepialus hecta* are greatly swollen and are almost filled with large unicellular

glands, each of which communicates with a spatula-shaped hair (Bertkau, 1882). In the male moth *Phassus schamyl* the hairs are scalelike with the distal end of each scale divided into two or three lobes (Deegener, 1905). The same kind of organ is found in the male moths *Syrichthus malvæ* and *Pechipogon barbalis* (Illig, 1902). In the latter species, instead of there being a tuft of hairs on each hind tibia, each front tibia bears three tufts. In the male moth *Sphinx convolvuli* a pair of lateral tufts of scalelike hairs is found at the proximal end of the abdomen (Tozzetti, 1870). In the female moths *Taumatopoca pinivora* and *Stilpnolia salicis* the scent-producing organ is a large paired tuft of hairs on both sides and above the anus (Freiling, 1909). In many male butterflies, the scent scales on the wings serve as scent-producing organs (Müller, 1877). Each scale is connected with a unicellular gland (Thomas, 1893; Illig, 1902). In the second type of scent-producing organ, the secretion from the gland cells passes into the hairs and scales and then spreads over their surfaces, whereby the odor from the secretion is more effectively disseminated.

In regard to storing the odor in an "evaginable" sac, the third type is a little farther advanced than the second type. In the male butterflies *Danais* and *Euplœa* the scent-producing organ consists of two large chitinous invaginated sacs, lined with scalelike hairs. One of these sacs lies on either side of the abdomen and opens between the seventh and eighth sterna (Illig, 1902). In the female butterfly *Gonopteryx rhamni* this organ is a single invaginated sac, but in the female of *Euplœa* it consists of a circle of scalelike hairs around the anus and of a pair of invaginated sacs, lined with hairs as usual (Freiling, 1909). Each hair is connected with a unicellular gland. The sacs are evaginated by blood pressure and retracted by muscles. It is thus seen that the odorous substance may be more or less retained in the invaginated sacs, but when the sacs are evaginated, like the fingers of a glove, all the odor escapes.

In regard to storing the secretion, the fourth type is more highly organized than any one of the preceding types of scent-producing organs. In the roach *Periplaneta orientalis* this organ consists of a pair of shallow pouches in the articular membrane between the fifth and sixth abdominal terga. The pouches are covered by the fifth tergum, but open to the exterior by a pair of slit-shaped openings. They are lined with hairs, each of which connects with a unicellular gland (Minchin, 1888). In the sexually matured male roach *Phyllodromia germanica* there are two double pouches, one

of which is located in the articular membrane between the fifth and sixth and the other between the sixth and seventh abdominal terga. These pouches are not lined with hairs. The tubes from the unicellular glands carry the secretion directly to the pouch where it is forced to the exterior by muscles constricting the lumen of the pouch (Oettinger, 1906). In the female moth *Orgyia antiqua* the scent-producing organ is a shallow pouch in the articular membrane between the eighth and ninth abdominal terga. The unicellular glands lie in groups like several bunches of grapes just beneath the thin membrane. Freiling (1909) saw no tubes connecting the gland cells with this membrane. He thinks that the secretion passes through the membrane by infiltration. In the petiole of the worker ant of *Myrmica rubra*, Janet (1898) found an invaginated chamber. At the bottom of the chamber may be seen the exits of the tubes which lead to the bunch of unicellular glands. He also found in the same ant two small groups of unicellular glands beneath the articular membrane between the ninth and tenth abdominal terga. These glands are also connected with tubes which run to the exterior. Both of these organs may possibly be scent-producing organs. The wax glands of young worker bees may also have such a function. Each of these unicellular glands is nothing more than a hypodermal cell modified for secreting a substance which passes through many minute pores in the thick chitin of the abdominal segment. After coming in contact with the external air the substance changes to wax. In *Apis* these glands lie beneath the second, third, fourth, and fifth abdominal sterna, in *Melipona* beneath the last four abdominal terga, in *Trigona* beneath the last five abdominal terga, but in *Bombus* beneath both the abdominal sterna and terga (Dreyling, 1906).

The scent-producing organ of the honey bee belongs to the fourth type, and it is probably the most highly developed organ of this type. Nasonoff thought that the chitinous tubes ran into the bottom of the canal, chiefly formed by the anterior portion of the articular membrane, instead of them uniting with the posterior wall of the canal. If they united with the bottom of the canal, they would materially affect the flexibility of the membrane. Zoubareff (1883) imagines that the gland cells in this organ of the bee secrete the little drops of liquid which bees are said to let fall when flying. He thinks that these drops represent the excess of water contained in freshly gathered nectar over that in ripened honey.

In regard to storing and discharging the secretion as a means of

defence, the fifth type of scent-producing organ is the most highly organized of all five types. For storing the secretion, the ear-wig has two pairs of reservoirs in the third and fourth abdominal terga (Vossler, 1890). Both sexes of walking-sticks have two straight, ribbonlike blind sacs which lie in the thorax (Scudder, 1876). The electric-light bug has two long cœcal tubes in the metathorax (Leidy, 1847). In another bug, *Pyrhocoris apterus*, the scent-producing organ is quite complicated. It has a specialized reservoir with a valve to prevent the escape of the secretion (Mayer, 1874). The male roaches *Periplaneta orientalis* and *P. americana* have, besides the scent-producing organ in the articular membrane already mentioned, anal glands which are highly organized (Bordas, 1901). The unicellular glands belonging to the anal glands of a beetle, *Blaps mortisaga*, are very similar in structure to those of the bee (Gilson, 1889). Many species of Carabidæ and Dytiscidæ have been studied by Dierckx (1899). He finds that all their anal glands are highly organized and that the secretion is produced by many unicellular glands which lie either in the tubes leading to the reservoir or lie a short distance from these tubes. All of the gland cells are quite similar in structure to those of the bee. A highly organized anal gland has also been found in a few ants (Forel, 1878).

From this brief outline, it is seen that scent-producing organs have already been found in many insects belonging to five orders. There is a wide variation in organization between the lowest type and the highest type. All of those organs belonging to the first four types are used in all probability for alluring purposes and as a means for recognition, while those of the fifth type are perhaps used only as a means of defence. Of the scent-producing organs used for recognition, that of the honey bee is probably the most highly organized.

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EXPLANATION OF PLATES XIX AND XX.

All figures, except diagrams Nos. 1 and 2, are from camera lucida drawings, made at the base of the microscope. Figures 5 to 16b were made by using a V and S4 ocular and a $\frac{1}{2}$ oil immersion. Each one of these drawings is enlarged 875 diameters.

ABBREVIATIONS.

<i>AcSt</i>	accessory parts of sting.
<i>Amp</i>	ampulla of gland cell.
<i>AntP</i>	anterior portion of articular membrane.
<i>ArtM</i>	articular membrane.
<i>Can</i>	canal.
<i>CanTu</i>	canal of chitinous tube.
<i>Ch</i>	chitin.
<i>ClS</i>	clear spot in cytoplasm of cell.
<i>DDph</i>	dorsal diaphragm.
<i>ECan</i>	end of canal.
<i>FC</i>	fat cell.
<i>GlC</i>	gland cell.
<i>Glo</i>	globule of cell.
<i>Gr</i>	groovelike indentation in chitin forming canal.
<i>H</i>	heart.
<i>Hr</i>	hair.
<i>HrS</i>	hair socket.
<i>Hyp</i>	hypodermis.
<i>HypC</i>	hypodermal cell.
<i>LInt</i>	large intestine.
<i>M</i>	muscle to move sting.
<i>Mal</i>	Malpighian tubules.
<i>CE</i>	œnocyte.
<i>PostP</i>	posterior portion of articular membrane.
<i>RadStr</i>	radial streak of ampulla.
<i>SInt</i>	small intestine.
<i>St</i>	sting.
<i>T</i>	trachea.
<i>Tu</i>	chitinous tubes of gland cells.
<i>TrA</i>	transparent area in ampulla.
<i>VDph</i>	ventral diaphragm.

a to *f* of figure 2.—*a*, small barbed hairs; *b*, small spinelike hairs; *c*, groovelike indentations on anterior portion of articular membrane; *d*, heavy and wide vein of chitin between anterior and posterior portions of articular membrane; *e*, heavy and narrow vein of chitin in posterior portion of articular membrane; *f*, location of gland cells.

a to *e* of figure 4.—*a*, comparatively thin and almost transparent chitin; *b*, narrow, thick and yellow band of chitin; *c*, thick, semitransparent band of chitin; *d*, wide, thick and opaque band of chitin; *e*, thick semitransparent chitin.

a to *b* of figure 9.—*a*, hypodermal cells, which later become gland cells, now broken loose from hypodermal layer; *b*, a later stage of same.

v, the heavy and wide vein of chitin shown in figure 2, *d*.

Fig. 1 has been placed in the text.

PLATE XIX.—Fig. 2.—Diagram of articular membrane spread out flat under a low-power lens, showing its superficial appearance, and looking through the posterior part (*PostP*) of membrane at a deeper focus may be seen gland cells and tubes as shown at *f*. The material used for figs. 2 to 7 inclusive was fresh and was stained slightly with a weak solution of methylin green.

Fig. 3.—A small portion of anterior part (*AntP*) of membrane from fig. 2, showing the groovelike indentations (*Gr*). $\times 700$.

Fig. 4.—A small portion of posterior part (*PostP*) of membrane from fig. 2, looking at inner side of chitin with strong transmitted light. Four gland cells (*GlC*) and many tubes (*Tu*) are shown. The tubes are twice too wide. $\times 275$.

Fig. 5.—Large live gland cell, showing its structure.

Fig. 6.—Large live œnocyte, showing its structure.

Fig. 7.—Small live fat cell, showing its structure.

Fig. 8.—Cross section of a small portion of posterior part (*PostP*) of membrane from fig. 2 at *f*, after treatment with caustic potash, showing that tubes (*Tu*) are chitinous.

PLATE XX.—Fig. 9.—Sagittal section through articular membrane of a 15-day-old worker pupa (counting from the time the egg was laid), showing origin of gland cells from hypodermal cells (*HypC*). All material used for figs. 9 to 16*b* was fixed and stained.

Fig. 10.—Same kind of section as fig. 9, from a 16-day-old worker pupa, showing (1) great increase in size of gland cells (*GIC*) within one day's time; (2) presence of tubes (*Tu*); (3) thin hypodermis (*Hyp*); and (4) presence of chitin (*Ch*).

Fig. 11*a-c*.—Origin of chitinous tube from a hypodermal cell. 11*a* is from a 15-day-old worker pupa, and 11*b* and 11*c* are from a 15½-day-old worker pupa.

Fig. 12.—Same kind of section as fig. 9, from a 17-day-old worker pupa, showing, as compared with fig. 10, (1) a slight increase in size of gland cells; (2) a thinner hypodermis; and (3) thicker chitin.

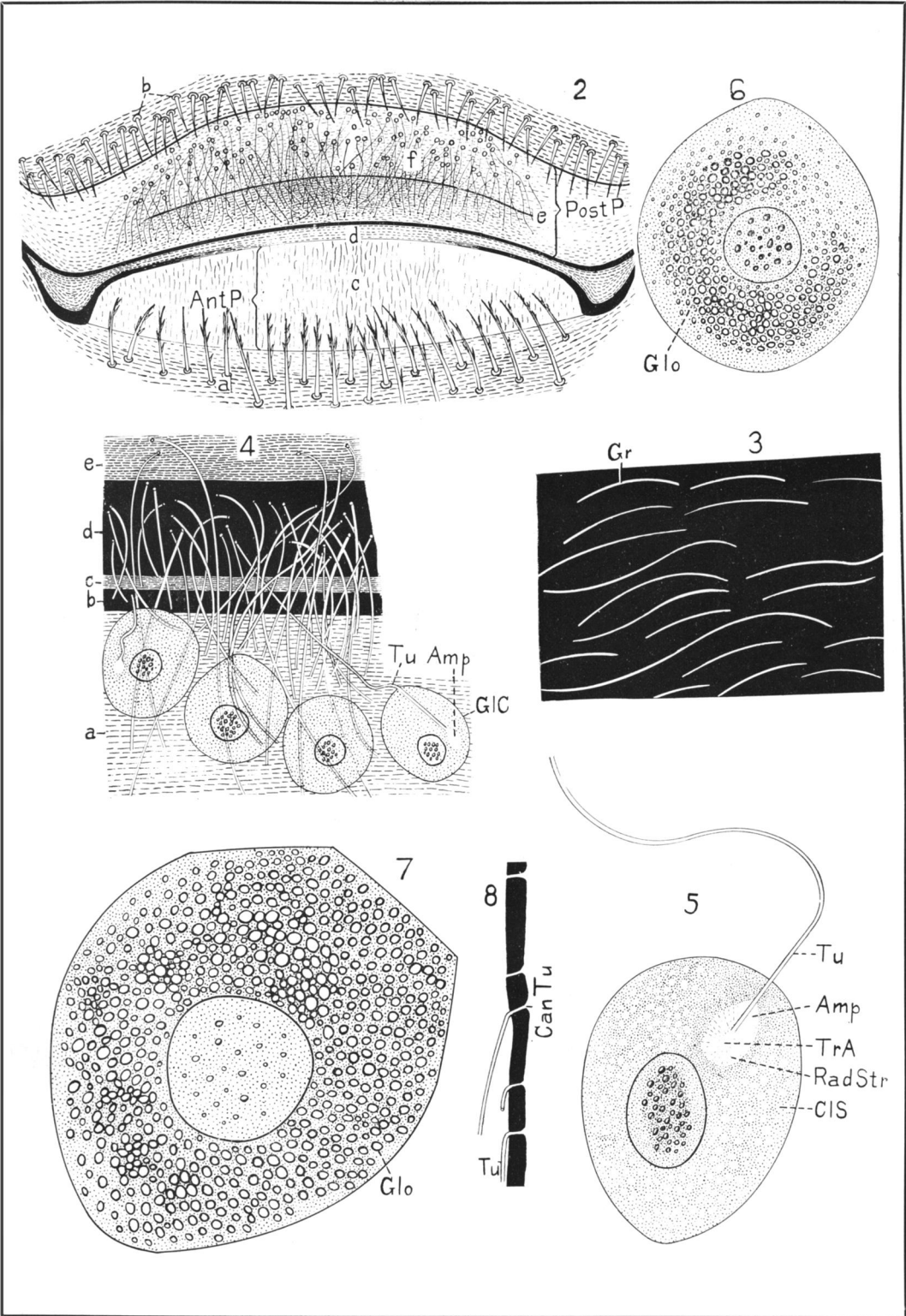
Fig. 13.—Same as fig. 12, but from a 19-day-old worker pupa, showing no noticeable change in size of gland cells.

Fig. 14.—Same as figs. 12 and 13, but from a 21-day-old worker pupa (now emerged as an imago insect), showing; (1) a considerable increase in size of gland cells, and (2) thicker chitin.

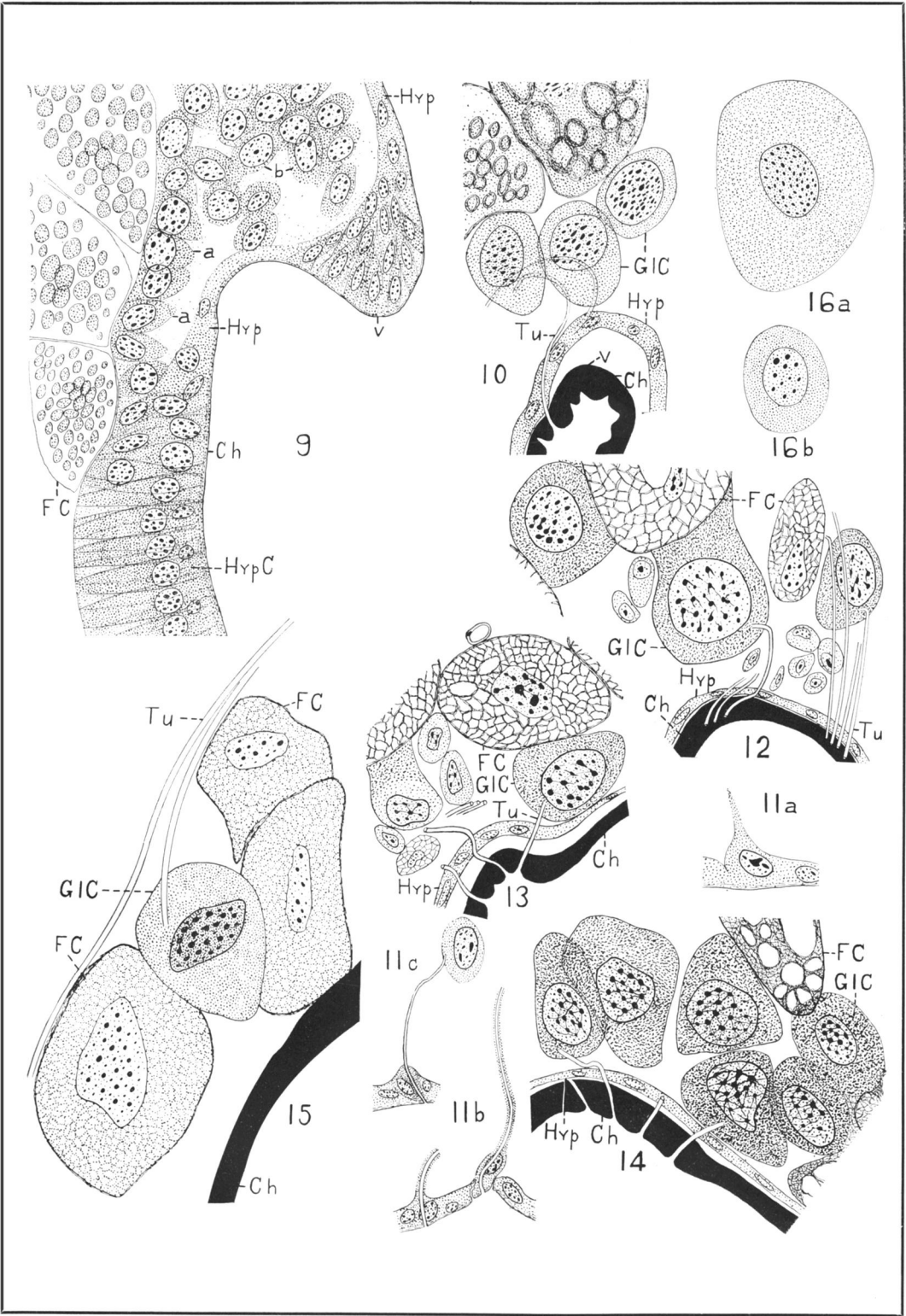
Fig. 15.—Same as fig. 14, but from an old worker bee, showing a still greater increase in size of gland cells. Compare this large gland cell, which was fixed and stained, with the large live gland cell in fig. 5.

Fig. 16*a*.—Large gland cell from an old queen.

Fig. 16*b*.—Large gland cell from a middle-aged pupal queen. Compare fig. 16*a* with gland cell in fig. 15.



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